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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Recombinant Herpes Viruses, a Vaccine Based on These
Recombinants, Their Preparation Process, Genes, Vectors
and Plasmids Used in This Process

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New recombinant herpes viruses, a vaccine based on these recombinants, their preparation process, genes, vectors and plasmids used in this process.

Abstract

The invention concerns the sequence of the unique short region Us of Marek's disease virus coding for the kinase protein. It also concerns the recombinant herpes viruses in which a heterologous gene has been inserted in the homologous region of the gene coding for the kinase protein and, in particular, the Marek recombinant viruses expressing a gene of an avian pathogenic agent which could possibly be a gene of another serotype of the Marek viruses. It also concerns a process for the preparation of these recombinants as well as the vaccines obtained.

New recombinant herpes viruses, a vaccine based on these recombinants, their preparation process, genes, vectors and plasmids used in this process.

The present invention concerns recombinant herpes viruses, in particular, for producing vaccines, their process of preparation and the plasmids produced during this process. Moreover, it concerns a part of the chromosome of Marek's disease virus (MDV) which can be used for preparing such vaccines.

10 Different types of viruses have been used as expression vectors of foreign genes, in particular of genes coding for antigenic proteins, and have proven their potential for immunizing animals. The vaccinia virus has, to a great extent, been used for constructing recombinant viruses. The herpes viruses have also been used: the herpes simplex virus (HSV) (M. Shih et al., Proc. Natl. Acad. Sci., USA, 1984, 81, 5867-5870), the varicella virus (VZV) (R. Lowe et al., Proc. Natl. Acad. Sci., USA, 1987, 84, 3896-3900). The foreign gene is inserted into a fragment of the genomic DNA of the herpes virus, corresponding to a non-essential region for the viral replication, cloned in a plasmid. This gene
20 is transferred into the viral genome by homologous recombination. This latter step is carried out by cotransfection of the herpes genomic DNA and plasmid since this genomic DNA is by nature infectious.

Different genes of herpes viruses have been identified as non-essential to viral growth, certain of these genes being associated with virulence.

- The gene of the thymidine kinase of the herpes simplex virus (D. Dubbs et al., Virology, 1965, 22, 493-502), of the Aujeszky virus (G. Tatarov, Zentralbl. Vet. Med., 1968, 15 B, 848853), of the rhinotracheal infectious bovine virus (S. Kit et al., Virology, 1983, 130, 381-389).
- The gene gIII of the Aujeszky virus (A. Robins et al., J. Virol., 1986, 59, 635-645).
- The gene gX of the Aujeszky virus (D. Thomsen et al., J. Virol., 1987, 61, 229-232).
- 10 - The gene gI of the Aujeszky virus (C. Mettenleiter et al., J. Virol., 1987, 61, 4030-4032).

Viruses in which one or other of these genes has been deleted nonetheless retain the capacity to produce a latent infection in mice.

Studies pertaining to the unique short region of the genome of the herpes simplex virus HSV-1 have been conducted (B. Megnier et al., Virology, 1988, 162, 251-254) and have shown that the viruses HSV-1 in the short region of which a gene has been deleted, have undergone an attenuation.

20 For their part, F.C. Purves et al. (Journal of Virology, 1987, vol. 61, No. 9, 2896-2901) have demonstrated that the open reading frame US3 of the short fragment of the HSV-1 virus genome codes for a virus enzyme, the kinase protein, and is not essential to the replication of said virus.

Studies have been undertaken on Marek's disease virus which belongs to the subfamily of gamma herpes viruses. This is an enveloped virus having a double stranded linear genomic DNA of

about 175 kilobases. Its genome is composed of a long segment (UL) and of a short segment (Us) framed by repeated inverted terminal sequences.

Marek's disease virus causes paralysis and a lymphoproliferative disease in chickens, usually, at the age of 2 to 5 months. This disease results in very significant economic losses (L. Payne, Biology of Marek's Disease Virus and the Herpes Virus of Turkeys, in The Herpes Virus, vol. 1, pp. 347-431, edited by B. Roizman, Plenum Press).

10 The strains of Marek's disease virus have been classified into three serotypes:

- serotype 1 comprises the pathogenic strains and attenuated strains derived therefrom.
- serotype 2 comprises the naturally attenuated strains.
- serotype 3 comprises the herpes virus of turkeys (HVT) and its variants.

Consequently, the term Marek's attenuated disease virus will designate serotypes 1, 2 and 3 at the same time.

20 Marek's disease virus (MDV) and herpes virus of turkeys (HVT) have similar genomic arrangements (A. Buckmaster et al., J. Gen. Virol., 1988, 69, 2033-2042) and numerous homologies of sequence all along their genome (C. Gibbs et al., Proc. Acad. Natl. Sci. USA, 1984, 81, 3365-3369).

The chicks are vaccinated at the age of one day and are then protected against Marek's disease for their entire life. For numerous years, vaccination with the herpes virus of live turkeys (HVT) has been very effective for controlling the disease.

Nevertheless, the emergence of new viral strains which are highly virulent has led to the use of strains of attenuated Marek's disease viruses of another serotype, to vaccinate and thus increase the level of protection, either, for example, the strain CVI 988, MDV serotype 1 attenuated by passing over cells (B. Rispens et al., Avian Dis., 1972, 16, 1108-125), or, for example, the association of the HVT MDV serotype 3 and SBI strains, MDV serotype 2 (K. Schat et al., J. Natl. Cancer Inst., 1987, 60, 1075-1082).

10 The genome of Marek's virus has certain similarities with the genome of alpha viruses, herpes simplex (HSV) and chickenpox (VZV). Most of the genes localized in the long region UL of the genome are approximately colinear between the herpes simplex virus, chickenpox (D. McGeogh, J. Gen. Virol., 1988, 69, 1531-1574) and Marek's disease virus (A. Buckmaster, 1988). Thus, in the international patent application WO 90/02802, it was proposed that the genes be inserted into this UL region of HVT and MDV.

20 On the other hand, the localization of genes in the Us segment shows a larger divergence between herpes viruses. Also among the dozen open reading frames identified for the herpes simplex virus, only four have a homology with the chickenpox virus (McGeogh, 1988, cited above).

 In fact, the prior art does not suggest that there is an interest in proceeding with a homology study of open reading frames of the unique short region of the genome of Marek's disease virus with the HSV-1 genes.

The present invention divulges, for the first time, the sequence coding for the kinase protein of the genome of Marek's disease virus (MDV) and allows one to establish, in a surprising manner, that this gene can be deleted without blocking the viral replication and allowing the insertion of heterologous sequences opening the way for development of a series of viral expression vectors.

10 This type of a recombinant and attenuated virus of Marek's disease constitutes a choice candidate for developing a viral vector expressing foreign genes to be used for polyvalent vaccination of poultry since it has the advantage of being able to be used for its own vaccinal properties and as a vaccine against other viral, bacterial and parasitic diseases as, for example, infectious avian bronchitis, Newcastle's disease, fowl plague, egg-drop syndrome, Gumboro's disease, chicken anaemia, coccidiosis, fowl pox, infectious laryngotracheitis, avian colibacillosis, is pasteurellosis, haemophilosis.

SUMMARY OF THE INVENTION

20 The object of the invention is to provide recombinant herpes viruses, including a recombinant attenuated Marek's disease virus (serotypes 1, 2 or 3), recombinant which can be used as a vaccine, the method for constructing such a recombinant virus as well as a vector virus allowing the multivalent vaccination against viral, bacterial or parasitic avian diseases.

One object of the invention is the nucleotide sequence

and its variants corresponding to the US3 gene which is homologous to the kinase protein gene of the herpes simplex virus and the surrounding regions. The term variants of the nucleotide sequence, as commonly used, means any equivalent sequence such as obtained, for example, by degeneration of the code, minor modifications, mutations or corresponding to viral variants.

Another object of the invention is a recombinant virus selected from the herpes viruses, including the viruses of pseudorabies disease, infectious bovine rhinotracheitis, equine rhinopneumonitis, feline rhinotracheitis, canine herpes.

Another object of the invention is also a Marek's disease recombinant virus comprising one or more heterologous genes inserted in the region of its genome corresponding to US3 gene in such a way so as to be expressed.

By "heterologous gene", one means, in particular, a gene coding for a protein or an immunogenic glycoprotein of a viral, bacterial or parasitic pathogenic agent, in particular, an agent associated with an avian pathology. This also relates to the construction of hybrid viruses, for example, by introducing, into the genome of a turkey herpes virus, genes coding for immunogenes of a Marek's disease virus of serotype 1 and/or serotype 2.

"Heterologous gene" is also intended to mean a gene coding for a peptide or a protein, for example, hormone, growth factor, immunomodulator.

The heterologous gene is preferably expressed under the control of regulating sequences of US3 gene transcription. One can, however, see to it that this expression is either controlled

by a promoting sequence coming from another gene of the virus in question, for example, the promoter of TK gene, of gA gene, of gB gene or from another herpes virus, for example, the promoter of gI gene of the infectious bovine rhinotracheitis virus or from gene II or gene III of pseudorabies virus.

Preferably, the start and stop codons of US3 gene are substituted by those of the gene to be expressed.

DESCRIPTION OF THE FIGURES

Figure 1 shows the construction of the plasmid pMDV 53L which contains the lacZ gene inserted instead of US3 gene.

Figure 2 shows the construction of the plasmid pMDV 53 CL which contains the lacZ gene under the control of the promoter iE of the human cytomegalovirus instead of US3 gene.

Figure 3 shows the plasmid pMDV 53F which contains the gene of the fusion protein of Newcastle's disease virus instead of US3 gene.

DESCRIPTION OF THE INVENTION: MATERIALS AND METHODS

Viral Strain

The serotype 1 strain RB1B of Marek's disease virus (MDV) was used (Schat K.A. et al., 1982, Avian Pathol. 11, 593-605).

The virus culture methods and methods for extraction of viral DNA having a high molecular weight have been described (C. Lee et al., 1980, J. Gen. Virol., 51, 235-253; N. Ross et al., 1989, 70, 1789-1804).

Cell Culture

The fibroblasts of chicken embryo (CEP) were cultivated in 199 F10, medium supplemented by penicillin, streptomycin, fungizone and fetal calf serum, as described (N. Ross, 1975, J. Gen. Virol., 28, 37-47).

Cloning of Viral DNA

Generally, the techniques used for the construction of recombinant plasmids are those described by T. Maniatis et al. (T. Maniatis et al., 1982, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

For all cloning and subcloning steps, the vector linearized by the appropriate restriction enzymes is dephosphorized before ligation. The purification of the DNA fragments starting from an agarose gel is done according to the technique described by the manufacturer: "GeneClean" (Bio 101, San Diego, California, USA).

Sequencing

The cloned fragments are sequenced according to the classic technique described by Sanger (G. Sanger, S. Nicklen, A. Coulson, 1977, Proc. Natl. Acad., USA 74, 5463-5467). The sequences after translation have been compared to the published sequences of herpes simplex virus and chickenpox (McGeogh, 1985, J. Mol. Biol. 181, 1, 13; K. Davison et al., 1986, J. Gen. Virol. 67, 1759-1816).

Controlled Mutagenesis

The DNA fragments, subcloned in the Blue Script vector (Stratagene, La Jolla, California, USA) are mutagenized after

separation of the DNAs simple fragment with the help of the R408 helper phage (Stratagene, La Jolla, California, USA) (M. Russel, S. Kidd, M. Kelley, 1986, Gene 45, 333-338).

The mutagenesis procedure and selection of the mutants by using the strain CJ 236 dut⁻, ung⁻ of E. coli (In Vitrogen, San Diego, California, USA) is described by T. Kunkel, (T. Kunkel 1985, Proc. Natl. Acad. Sci., 82, 488-492 and T. Kunkel et al., 1987, Methods of Enzymology 154, 367-382, Acad. Press).

In vivo Recombination

10 The recombinant viruses are obtained according to the conventional techniques of transfection of the sensitive cells, such as the calcium phosphate method or the one using the Lipofectine reactive described by the manufacturer BRL (P.L. Felgner et al., 1987, Proc. Natl. Acad. Sci., USA, 84, 7413). For this, the chicken embryo fibroblasts, cultivated to confluence, are cotransfected with the genomic DNA and the plasmid carrier of the DNA fragment to be inserted, flanked in 5' and 3' by the genome sequences which allows the recombination.

20 The recombinant viruses can then be screened by hybridization with an appropriate probe or by plaque colouring. When a gene marker, the lacZ gene of β -galactosidase, is inserted into the gene of Marek's disease virus, the expression of this gene can be followed by adding, to the cell covering, an agarose overcoat enclosing the chromogenic substrate for the β -galactosidase, e.g. Xgal (5-bromo-4-chloro-3-indolyl, B.D. galactopyranoside).

Example 1: Isolating an EcoRI fragment of 5.25 kilobases.

The viral genomic DNA was digested by the restriction enzyme EcoRI and the fragments cloned in the vector pUC 13 (Pharmacia) (Yannisch, Perron et al., 1985, Gene 33, 103-119).

Among the cloned fragments, a fragment of 5.25 kilobases, localized at the level of the small fragment Us, has been, more particularly, analyzed by sequencing (pMDV 05; sequence ID no. 1).

The sequence comprises six open reading frames (ORF).

- 10 The translated sequences of 4 of these ORF have a homology with the type I HSV virus proteins, localized in the Us fragment. In particular, the US3 gene of Marek's disease virus has a homology with the gene of the kinase protein of the herpes simplex virus.

Surprisingly, the study of this region has shown that the US3 gene can be deleted without blocking the viral replication.

Example 2: Construction of a plasmid pMDV 53L for which the US3 gene has been replaced by the lacZ gene (Figure 1).

- 20 The EcoRI fragment of 5.25 kilobases stemming from the clone pMDV 05 was digested by the NcoI enzyme and the extremities thus generated restored by the DNA polymerase Klenow fragment. It was then digested by the KpnI enzyme and the fragment of 1989 pairs of bases thus liberated was cloned in the Blue Script vector linearized by the enzymes EcoRV and KpnI to give the plasmid pMDV

52 having 4947 pairs of bases.

The NcoI and SalI sites were respectively introduced to the extremities 5' and 3' of the cloned fragment by controlled mutagenesis using the oligonucleotides designated by Seq ID no. 2 and 3, in the list of attached sequences, which generates the pMDV 53 plasmid. The lacZ gene was purified from the pMC 1871 plasmid (Pharmacia LKB, Uppsala, Sweden) (S.K. Shamira et al. 1983, Gene 25, 71-82) by digestion by the enzymes SmaI and SalI.

10 It was then inserted into the pMDV 53 plasmid, partially digested by the NcoI enzyme, treated by the DNA polymerase Klenow fragment and then controlled by the enzyme SalI, which generates the plasmide pMDV 53L of about 6687 pairs of bases.

Example 3: Preparation of a Marek's disease virus comprising the lacZ gene.

20 The chicken embryo fibroblasts were cotransfected with the total chromosomal DNA of the virus and the DNA of the linearized plasmid pMDV 53L (10 to 50 µg). The cultures were observed for 4 to 6 days until infectious centres appeared. Alternatively, the cells were trypsinated after 72 hours, then reinoculated (1:1 or 1:2) in a secondary passage, until lysis segments were obtained.

The medium was then replaced by the new medium comprising 1% agarose and 0.5% Xgal.

The plaques which are due to the recombination viruses are distinguished by their blue colour.

The viruses can thus be purified by plaque purification and, after inoculation with healthy cells, give cytopathogenic effect shapes coloured in blue in the presence of Xgal.

Example 4: Construction of the plasmid pMDV 53 CL which comprises the lacZ gene under the control of the immediate early promoter of the human cytomegalovirus (CMV) (Figure 2).

The lacZ gene of β -galactosidase was placed under the control of the immediate early promoter of the human cytomegalovirus (IECMV) in the vector pCMV-lacZ.

10 The fragment of about 4500 pairs of bases comprising the whole, the promoter IE of the cytomegalovirus and the lacZ gene, were digested by the EcoRI enzyme and the extremities filled by the DNA polymerase Klenow fragment. It was then digested by the SalI enzyme.

The fragment thus liberated was cloned in the partially digested pMDV 53 vector, by the NcoI enzyme, treated by the DNA polymerase Klenow fragment and then digested by the SalI enzyme. This plasmid composed of about 8200 pairs of bases is called pMDV 53 CL.

20 Example 5: Construction of a Marek recombinant virus for which the lacZ gene was introduced under control of the immediate early promoter of the cytomegalovirus, instead of the US3 gene.

The CEP were cotransfected with the genomic DNA of the virus and from 10 to 50 μ g of DNA of the linearized plasmid pMDV 53 CL.

One can thus obtain the recombinant viruses which are distinguished by the appearance of blue-coloured infectious plaques in the presence of the chromogen substrate Xgal. These viruses were purified according to the plaque purification technique and allowed to infect the secondary chicken embryo fibroblasts.

The blue-coloured infectious plaques can be obtained in the presence of Xgal which shows that the lacZ gene is inserted at the locus of the US3 gene.

- 10 Example 6: Construction of the pMDV 53F plasmid which comprises the gene of the fusion protein of Newcastle's disease virus instead of the US3 gene (Figure 3).

The fusion gene (J. Taylor et al., 1990, J. Virol., 64, 1441-1450) was introduced in the form of a fragment at the blunt ends in the Blue Script vector at the SmaI site to give the pNF1 plasmid having 5300 pairs of bases.

- 20 NcoI and SalI sites were introduced by controlled mutagenesis at the level of the ATG and stop codons of the fusion gene, due to the oligonucleotides indexed SEQ ID no. 4 and SEQ ID no. 5 respectively in the list of the attached sequences.

The fragment of 1682 pairs of bases NcoI/SalI coming from the pNF2 plasmid was inserted into the pMDV 53 vector partially digested by the NcoI enzyme and digested by the SalI enzyme to give the pMDV 53F plasmid having 5369 bases pairs.

Example 7: Construction of a Marek's disease virus comprising the fusion protein gene of Newcastle's disease virus.

The chicken embryo fibroblasts were cotransfected with the total genomic DNA of the virus and 10 to 50 μ g of linearized DNA of the pMDV 53F plasmid. The cultures were observed for the appearance of infectious plaques.

The recombinant viruses were then screened by hybridization with a probe including the fusion gene.

- 10 Similar procedures were used for the construction of the non-avian recombinant herpes viruses, by inserting a heterologous gene in the Us region and, in particular, in the homologous gene at US3 gene of Marek's virus.

The invention also concerns vaccines, live or not, made up of or containing recombinant viruses constructed according to the invention, or containing immunogenes expressed by these viruses.

APPENDIX I

LIST OF SEQUENCES

SEQ ID no. 1

Length of sequence: 5,255 pairs of bases
 Type of molecule sequenced: genomic DNA
 Origin of the molecule: Marek's disease virus,
 strain RBL B
 Experimental source: PMDV 05 plasmid

Characteristics:

from 1 to 324 pairs of bases: non-coding region
 from 325 to 1,135 pairs of bases: US1 gene. The gene is
 coded by the complementary DNA segment at
 the indicated sequence and is transcribed
 from right to left (SEQ ID no. 1.B)
 from 623 to 1,214 pairs of bases: US2 gene
 from 1,215 to 1,245 pairs of bases: non-coding region
 from 1,246 to 2,451 pairs of bases: US3 gene
 from 2,452 to 2,563 pairs of bases: non-coding region
 from 2,564 to 3,004 pairs of bases: US4 gene
 from 3,005 to 3,190 pairs of bases: non-coding region
 from 3,191 to 4,384 pairs of bases: US5 gene
 from 4,385 to 4,494 pairs of bases: non-coding region
 from 4,495 to 5,253 pairs of bases: US6 gene

CAAAAATTACATTAGTAATCTTTTCGGTGGCTTACCAAAATCGTCTCTTGGTATATCCATATCATCGAAC 72
 ATTGTAGCATTGACTCTGCTCATCGTTGTCTTTCAAAATCGCTCGATTGTGTAATCTCTCTGATGTTAGAA 144
 GTATATGGAAGATAGCTGGATACATAAGTGATCATAGAAGGGTTTGTATTGCAAGTAATACAAATTATAC 216
 GTGACACTATAGCGACGGTGTAGCGATGCACCTAATCGTAATGTGTATACGCCCATCATGTAAATTATATC 288
 TAATTGGTAGCAAGTAGGTCTGTGCAATAACAGCTAATGACTACCGGCTCTACATTTTTCTGTATTCTGTA 360
 CTTCTCTGTCGAGTGTAAACGAACCGGAATGCAATCGCATCTCTATCTCTTTCTTGCACATTTTCCACA 432
 ACASATAATCTGCCGGTGTACTACTCATTTGAGGTGGTTCGATTTCGGAGGTTTAGAGGATTGGGTGG 504
 GGACCGAGGATTTTGTATACACATACCATCTCACTGTCCGAAAAATCGCTCTATCTCTGGGGTGTGCAA 576
 CTTCCGGTCCCATGTAGATGTCAAGAGAGTTTGAATTTGTCCGGA ATG GCC CAC GGC ATA CCG 640
 Met Ala His Gly Ile Pro 6
 GAC CAG GTC CCA GAC ACT TTG ATT GCA AGT AAC CTT TTT GGC AAA GGA ATA CAT 694
 Asp Gln Val Pro Asp Thr Leu Ile Ala Ser Asn Leu Phe Gly Lys Gly Ile His 24
 TCG AGC GCA ATG CGA CAT ATA TCT GCC GCC CCA ACT ATC CAC AAG CTA TGT GGA 748
 Ser Ser Ala Met Arg His Ile Ser Ala Ala Pro Thr Ile His Lys Leu Cys Gly 42
 GCA TTA CCA GAA ACT TCA GAT TCC AAC ATC AAA TAT CCA GAT AGA ACA TCC TGC 802
 Ala Leu Pro Gly Thr Ser Asp Ser Asn Ile Lys Tyr Pro Asp Arg Thr Ser Cys 60
 CAT TCT GTG GAA CAT CCT GCA ACA TCT TCA AAT AGC CGC ACT ATA AAC GAA TCC 856
 His Ser Val Glu His Pro Ala Thr Ser Ser Asn Ser Arg Thr Ile Asn Gly Ser 78
 CTA GTT CCG GCC AAT CCG GTA CCA CGA ACT CCA GTT CCA TCT GGT GGC TTT GTC 910
 Leu Val Pro Ala Asn Pro Val Pro Arg Thr Pro Val Pro Ser Gly Gly Phe Val 96
 CTT ACT ATC GGT CGA TGT TGC CGA GGA AGA ATT AAC ATG GGT TGT GCA AAA CCG 964
 Leu Thr Ile Gly Arg Cys Cys Arg Gly Arg Ile Asn Met Gly Leu Ala Lys Arg 114
 AAT AGG TCT GCA GCT CTG ACG ATT ATG GGC ACA CCC ACA TCA TCC TGT ATT TGT 1018
 Asn Arg Ser Ala Ala Leu Thr Ile Met Gly Thr Pro Thr Ser Ser Cys Ile Cys 132
 TCC ATA CAT TGC TTT ATA AGG AAT ATC CAT AAA GTA GAT GCA GCA TCT CTA GAT 1072
 Ser Ile His Cys Phe Ile Arg Asn Ile His Lys Val Asp Ala Ala Ser Leu Asp 150
 CTT CCT GGC AAT CGA TCG CAT TCA TCT AGA AGT GTG ACT ATA GTT ATC ATG GAC 1126
 Leu Pro Gly Asn Arg Ser His Ser Ser Arg Ser Val Thr Ile Val Ile Met Asp 168
 ACA CCC ATC TTC ACT CCA CCA ATA ATC TTT TTT ATT GTT AAT AAC TGG GCC GGT 1180
 Thr Pro Ile Phe Thr Pro Pro Ile Ile Phe Phe Ile Val Asn Asn Trp Ala Gly 186
 CTG ATC TCC AAA TCT TAT ACC TCT GGT AGA ATA TGAACAGGGTTAAACTAGTAATAG 1260
 Leu Ile Thr Lys Ser Tyr Thr Ser Gly Arg Ile 197
 ACTGGATG TCT TCG AGT CCG BAG GCA GAA ACG ATG GAA TGC GGC ATT TCT TCG TCG 1296
 Met Ser Ser Ser Pro Gly Ala Gly Thr Met Gly Cys Gly Ile Ser Ser Ser 214
 AAA GTA CAC GAC TCT AAA ACT AAT ACT ACC TAC GGA ATT ATA CAT AAC AGC ATC 1350
 Lys Val His Asp Ser Lys Thr Asn Thr Thr Tyr Gly Ile Ile His Asn Ser Ile 232
 AAT GGT ACG GAT ACG ACG TTG TTT GAT ACT TTT CCC GAC AGT ACC GAT AAC GCG 1404
 Asn Gly Thr Asp Thr Thr Leu Phe Asp Thr Phe Pro Asp Ser Thr Asp Asn Ala 250
 GAA GTG ACG GCG GAT GTG GAC GAT GTG AAG ACT GAG AGC TCT CCC GAG TCC CAA 1458
 Gly Val Thr Gly Asp Val Asp Asp Val Lys Thr Gly Ser Ser Pro Gly Ser Gln 268

2050350

TCT GAA GAT TTG TCA CCT TTT GGG AAC GAT GGA AAT GAA TCC CCC GAA ACG GTG 1512
 Ser Glu Asp Leu Ser Pro Phe Gly Asn Asp Gly Asn Glu Ser Pro Glu Thr Val 286
 ACG GAC ATT GAT GCA GTT TCA GCT GTG CGA ATG CAG TAT AAC AAT GTT TCA TCG 1566
 Thr Asp Ile Asp Ala Val Ser Ala Val Arg Met Gln Tyr Asn Asn Val Ser Ser 304
 TTA TCG CCC GGA TCT GAA GGG TAT ATC TAT GTT TGT ACA AAG CGT GGG GAT AAT 1620
 Leu Ser Pro Gly Ser Glu Gly Tyr Ile Tyr Val Cys Thr Lys Arg Gly Asp Asn 322
 ACC AAG AGA AAA GTC ATT GTG AAA GCT GTG ACT GGT GAC AAA ACC CTT GGG AGT 1674
 Thr Lys Arg Lys Val Ile Val Lys Ala Val Thr Gly Asp Lys Thr Leu Gly Ser 340
 GAA ATT GAT ATA TTA AAA AAA ATG TCT CAC CGC TCC ATA ATT AGA TTA GTT CAT 1728
 Glu Ile Asp Ile Leu Lys Lys Met Ser His Arg Ser Ile Ile Arg Leu Val His 358
 GCT TAT AGA TGG AAA TCG ACA GTT TGT ATG GTA ATG CCT AAA TAC AAA TGC GAC 1782
 Ala Tyr Arg Trp Lys Ser Thr Val Cys Met Val Met Pro Lys Tyr Lys Cys Asp 376
 TTG TTT ACG TAC ATA GAT ATC ATG GGA CCA TTG CCA CTA AAT CAA ATA ATT ACG 1836
 Leu Phe Thr Tyr Ile Asp Ile Met Gly Pro Leu Pro Leu Asn Gln Ile Ile Thr 394
 ATA GAA CGG GGT TTG CTT GGA GCA TTG GCA TAT ATC CAC GAA AAG GGT ATA ATA 1890
 Ile Glu Arg Gly Leu Leu Gly Ala Leu Ala Tyr Ile His Glu Lys Gly Ile Ile 412
 CAT CGT GAT GTA AAA ACT GAA AAT ATA TTT TTG GAC AAA CCT GAA AAT GTA GTA 1944
 His Arg Asp Val Lys Thr Glu Asn Ile Phe Leu Asp Lys Pro Glu Asn Val Val 430
 TTG GGG GAC TTT GGG GCA CCA TGT AAA TTA GAT GAA CAT ACA GAT AAA CCC AAA 1998
 Lys Gly Asp Phe Gly Ala Ala Cys Lys Leu Asp Gly His Thr Asp Lys Pro Lys 446
 TGT TAT GGA TGG AGT GGA ACT CTG GAA ACC AAT TCG CCT GAA CTG CTT GCA CTT 2052
 Cys Tyr Gly Trp Ser Gly Thr Leu Glu Thr Asn Ser Pro Glu Leu Leu Ala Leu 466
 GAT CCA TAC TGT ACA AAA ACT GAT ATA TGG AGT GCA GGA TTA GTT CTG TTT GAG 2106
 Asp Pro Tyr Cys Thr Lys Thr Asp Ile Trp Ser Ala Gly Leu Val Leu Phe Glu 484
 ATG TCA GTA AAA AAT ATA ACC TTT TTT GGC AAA CAA GTA AAC GGC TCA GGT TCT 2160
 Met Ser Val Lys Asn Ile Thr Phe Phe Gly Lys Gln Val Asn Gly Ser Gly Ser 502
 CAG CTG AGA TCC ATA ATT AGA TGC CTG CAA GTC CAT CCG TTG GAA TTT CCA CAG 2214
 Gln Leu Arg Ser Ile Ile Arg Cys Leu Gln Val His Pro Leu Glu Phe Pro Gln 520
 AAC AAT TCT ACA AAC TTA TGC AAA CAC TTC AAG CAG TAC GCG ATT CAG TTA CGA 2268
 Asn Asn Ser Thr Asn Leu Cys Lys His Phe Lys Gln Tyr Ala Ile Gln Leu Arg 538
 CAT CCA TAT GCA ATC CCT CAG ATT ATA CGA AAG AGT GGT ATG ACG AGT GAT CTT 2322
 His Pro Tyr Ala Ile Pro Gln Ile Ile Arg Lys Ser Gly Met Thr Met Asp Leu 556
 GAA TAT GCT ATT ACA AAA ATG CTC ACA TTC GAT CAG GAG TTT AGA CCA TCT GCC 2376
 Glu Tyr Ala Ile Ala Lys Met Leu Thr Phe Asp Gln Glu Phe Arg Pro Ser Ala 574
 CAA GAT ATT TTA ATG TTG CCT CTT TTT ACT AAA GAA CCC GCT GAC GCA TTA TAC 2430
 Gln Asp Ile Leu Met Leu Pro Leu Phe Thr Lys Glu Pro Ala Asp Ala Leu Tyr 592
 ACG ATA ACT GCC GCT CAT ATG TAAACACCCGTCAAAATAACTTCAATGATTCATTTATAATA 2494
 Thr Ile Thr Ala Ala His Met 599
 TATACTACGGTTACCTGCAATAATGACACCAATTCGAAGTCTTTGGAAGATTCGACACCTTTTTCGCAATG 2566
 Met 600
 GCA CCT TCG GGA CCT ACG CCA TAT TCC CAC AGA CCG CAA ATA AAG CAT TAT GGA 2620
 Ala Pro Ser Gly Pro Thr Pro Trp Ser His Arg Pro Gln Ile Lys His Tyr Gly 618
 ACA TTT TTG GAT TGC ATG AGA TAT ACT CTA AAC GAT GAG AGT AAG GTA GAT GAT 2674
 Thr Phe Leu Asp Cys Met Arg Tyr Thr Leu Asn Asp Gly Ser Lys Val Asp Asp 636

AGA TGT TCA GAC ATA CAT AAC TCC TTA GCA CAA TCC AAT GTT ACT TCA AGC ATG 2728
 Arg Cys Ser Asp Ile His Asn Ser Leu Ala Glu Ser Asn Val Thr Ser Ser Met 654
 TCT GTA ATG AAC GAT TCG GAA GAA TAT CCA TTA ATA AAT GGA CCT TCG ATG CAG 2781
 Ser Val Met Asn Asp Ser Glu Glu Tyr Pro Leu Ile Asn Gly Pro Ser Met Glu 672
 GCA GAG GAC CCT AAA AGT GTT TTT TAT AAA GTT CGT AAG CCT GAC CGA AGT GGT 2836
 Ala Glu Asp Pro Lys Ser Val Phe Tyr Lys Val Arg Lys Pro Asp Arg Ser Arg 690
 GAT TTT TCA TGG CAA AAT CTG AAC TCC CAT GGC AAT AGT GGT CTA CGT CGT GAA 2890
 Ala Glu Asp Pro Lys Glu Asn Leu Asn Ser His Gly Asn Ser Gly Leu Arg Arg Glu 708
 Asp Phe Ser Trp Glu Asn Leu Asn Ser His Gly Asn Ser Gly Leu Arg Arg Glu 2944
 AAA TAT ATA CGT TCC TCT AAG AGG CGA TGG AAG AAT CCC GAG ATA TTT AAG GTA 726
 Lys Tyr Ile Arg Ser Ser Lys Arg Arg Trp Lys Asn Pro Glu Ile Phe Lys Val 2998
 TCT TTG AAA TGT GAA TCA ATT GGC GCT GGT AAC GGA ATA AAA ATT TCA TTC TCA 744
 Ser Leu Lys Cys Glu Ser Ile Gly Ala Gly Asn Gly Ile Lys Ile Ser Phe Ser 2998
 TTT TTC TAACATTATAATATATCAGATCGTTTCTTATATACITATTTTCATCGTCGGGATATGACTAAC 3067
 Phe Phe 746
 GTATACAAAGTTACAAGAAACAACCTGCTTAACGTGCAACATAACGGAATAAAAATATATATAGCGTCTCT 3139
 ATAANTGTTATATTGGCACCTTTTAGAGCTTCGGTATGAATAGATACAGATAG AAA GTA TTT TTT 3205
 Met Lys Val Phe Phe 751
 TTT AGA TAT ATC TCA TCC ACG AGA ATG ATT CIT ATA ATC TGT CTA CIT TTG GGA 3259
 Phe Arg Tyr Ile Ser Ser Thr Arg Met Ile Leu Ile Ile Cys Leu Leu Leu Gly 769
 ATT GGG GAC ATG TCC GCA ATG GGA CTT AAG AAA GAC AAT TCT CCG ATC ATT CCC 3313
 Ile Gly Asp Met Ser Ala Met Gly Leu Lys Lys Asp Asn Ser Pro Ile Ile Pro 787
 ACA TTA CAT CCG AAA GGT AAT GAA AAC CTC CGG GCT ACT CTC AAT GAA TAC AAA 3367
 Thr Leu His Pro Lys Gly Asn Glu Asn Leu Arg Ala Thr Leu Asn Glu Tyr Lys 805
 ATC CCG TCT CCA CTG TTT GAT ACA CTT GAC AAT TCA TAT GAG ACA AAA CAC GTA 3421
 Ile Pro Ser Pro Leu Phe Asp Thr Leu Asp Asn Ser Tyr Glu Thr Lys His Val 823
 ATA TAT ACG GAT AAT TGC AGT TTT GCT GTT TTG AAT CCA TTT GGC GAT CCG AAA 3475
 Ile Tyr Thr Asp Asn Cys Ser Phe Ala Val Leu Asn Pro Phe Gly Asp Pro Lys 841
 TAT ACG CTT CTC AGT TTA CTG TTG ATG GGA CGA CGC AAA TAT GAT GCT CTA GTC 3529
 Tyr Thr Leu Leu Ser Leu Leu Leu Met Gly Arg Arg Lys Tyr Asp Ala Leu Val 859
 GCA TGG TTT GTC TTG GGC AGA GCA TGT GGG AGA CCA ATT TAT TTA CGT GAA TAT 3583
 Ala Trp Phe Val Leu Gly Arg Ala Cys Gly Arg Pro Ile Tyr Leu Arg Glu Tyr 877
 GCC AAC TGC TCT ACT AAT GAA CCA TTT GGA ACT TGT AAA TTA AAG TCC CTA GGA 3637
 Ala Asn Cys Ser Thr Asn Glu Pro Phe Gly Thr Cys Lys Leu Lys Ser Leu Gly 895
 TGG TGG GAT AGA AGA TAT GCA ATG ACG AGT TAT ATC GAT CGA GAT GAA TTG AAA 3691
 Trp Trp Asp Arg Arg Tyr Ala Met Thr Ser Tyr Ile Asp Arg Asp Glu Leu Lys 913
 TTG ATT ATT GCA GCA CCC AGT CGT GAG CTA AGT GGA TTA TAT ACG CGT TTA ATA 3745
 Leu Ile Ile Ala Ala Pro Ser Arg Glu Leu Ser Gly Leu Tyr Thr Arg Leu Ile 931
 ATA ATT AAT GGA GAA CCC ATT TCG AGT GAC ATA TTA CTG ACT GTT AAA GAA ACA 3799
 Ile Ile Asn Gly Glu Pro Ile Ser Ser Asp Ile Leu Leu Thr Val Lys Glu Thr 949
 TGT AGT TTT TCG AGA CGG GGG ATA AAG GAT AAC AAA CTA TGC AAA CCG TTC AGT 3853
 Cys Ser Phe Ser Arg Arg Arg Gly Ile Lys Asp Asn Lys Leu Cys Lys Pro Phe Ser 967
 TTT TTT GTC AAT GGT ACA ACA CGG CTG TTA GAC ATG GTG GGA ACA GGA ACC CGG 3907
 Phe Phe Val Asn Gly Thr Thr Arg Leu Leu Asp Met Val Gly Thr Gly Thr Pro 985
 AGA GCT CAT GAA GAA AAT GTG AAG CAG TGG CTT GAA CGA ATT GGT GGT AAA CAT 3761

Arg Ala His Glu Glu Asn Val Lys Gln Trp Leu Glu Arg Ile Gly Gly Lys His 1003
 CTA CCA ATC GTC GTC GAA CAA TCT ATG CAA CAA GTC TCA AAT TTG CCG AGA AGT 4015
 Leu Pro Ile Val Val Glu Thr Ser Met Gln Gln Val Ser Asn Leu Pro Arg Ser 1021
 TTT AGA GAT TCA TAT TTC AAA TCA CCT GAC GAC GAT AAA TAT GAT GAC GTC AAA 4069
 Phe Arg Asp Ser Tyr Phe Lys Ser Pro Asp Asp Lys Tyr Asp Asp Val Lys 1039
 ATG ACA TCG GCC ACT ACT AAT AAC ATT ACC ACC TCC GTG GAT GGT TAC ACT GGA 4123
 Met Thr Ser Ala Thr Thr Asn Asn Ile Thr Ser Val Asp Gly Tyr Thr Gly 1057
 CTC ACT AAT CGG CCC GAG GAC TTT GAG AAA GCA CCA TAC ATA ACT AAA CGA CCG 4177
 Leu Thr Asn Arg Pro Glu Asp Phe Glu Lys Ala Pro Tyr Ile Thr Lys Arg Pro 1075
 ATA ATC TCT GTC GAG GAG GCA TCC AGT CAA TCA CCT AAA ATA TCA ACA GAA AAA 4231
 Ile Ile Ser Val Glu Glu Ala Ser Ser Gln Ser Pro Lys Ile Ser Thr Glu Lys 1093
 AAA TCC CGA ACG CAA ATA ATA ATT TCA CTA GTT GTT CTA TGC GTC ATG TTT TGT 4285
 Lys Ser Arg Thr Gln Ile Ile Ile Ser Leu Val Val Leu Cys Val Met Phe Cys 1111
 TTC ATT GTA ATC GGG TCT GGT ATA TGG ATC CTT CGC AAA CAC CGC AAA ACG GTG 4339
 Phe Ile Val Ile Gly Ser Gly Ile Trp Ile Leu Arg Lys His Arg Lys Thr Val 1129
 ATG TAT GAT AGA CGT CGT CCA TCA AGA CGG GCA TAT TCC CGC CTA TAACACGTGTT 4395
 Met Tyr Asp Arg Arg Arg Pro Ser Arg Ala Tyr Ser Arg Leu 1144
 TGGTATGGCGGTGTCGCTATAGTCATAGAAGATTGACTACATTGCATCAATGACATTATATAGCTTCTTTG 4467
 GTCAGATAGACGGCGTGTGTGATGCGATG TAT CTA CTA CAA TTA TTA TTT TGG ATC CGC 4527
 Met Tyr Leu Leu Gln Leu Leu Phe Trp Ile Arg 1155
 CTC TTT CGA GGC ATC TGG TCT ATA GTT TAT ACT GGA ACA TCT GTT ACG TTA TCA 4581
 Leu Phe Arg Gly Ile Trp Ser Ile Val Tyr Thr Gly Thr Ser Val Thr Leu Ser 1173
 ACG GAC CAA TCT GCT CTT GTT GCG TTC TGC GGA TTA GAT AAA ATG GTG AAT GTA 4635
 Thr Asp Gln Ser Ala Leu Val Ala Phe Cys Gly Leu Asp Lys Met Val Asn Val 1191
 CGC GGC CAA CTT TTA TTC CTG GGC GAC CAG ACT CGG ACC AGT TCT TAT ACA GGA 4689
 Arg Gly Gln Leu Leu Phe Leu Gly Asp Gln Thr Arg Thr Ser Ser Tyr Thr Gly 1209
 ACG ACG GAA ATC TTG AAA TGG GAT GAA GAA TAT AAA TGC TAT TCC GTT CTA CAT 4743
 Thr Thr Glu Ile Leu Lys Trp Asp Glu Glu Tyr Lys Cys Tyr Ser Val Leu His 1227
 GCG ACA TCA TAT ATG GAT TGT CCT GCT ATA GAC GCC ACG GTA TTC AGA GGC TGT 4797
 Ala Thr Ser Tyr Met Asp Cys Pro Ala Ile Asp Ala Thr Val Phe Arg Gly Cys 1245
 AGA GAC GCT GTG GTA TAT GCT CAA CCT CAT GAT AGA GTA CAA CCT TTT CCC GAA 4851
 Arg Asp Ala Val Val Tyr Ala Gln Pro His Asp Arg Val Gln Pro Phe Pro Glu 1263
 AAG GGA ACA TTG TTG AGA ATT GTC GAA CCC AGA GTA TCA GAT ACA GGC AGC TAT 4905
 Lys Gly Thr Leu Leu Arg Ile Val Glu Pro Arg Val Ser Asp Thr Gly Ser Tyr 1281
 TAC ATA CGT GTA GCT CTC GCT GGA AAT ATG AGC GAT ATA TTT AGA ATG GCT 4959
 Tyr Ile Arg Val Ala Leu Ala Gly Arg Asn Met Ser Asp Ile Phe Arg Met Ala 1299
 GTT ATT ATA AGG AGT AGC AAA TCT TGG GCN TGT AAT CAC TCT GCT AGT TCA TTT 5013
 Val Ile Ile Arg Ser Ser Lys Ser Trp 22 Cys Asn His Ser Ala Ser Ser Phe 1317
 CAG GCC CAT AAA TGT ATT CGC TAT GTC GAC CGT ATG GCC TTT GAA AAT TAT CTG 5067
 Gln Ala His Lys Cys Ile Arg Tyr Val Asp Arg Met Ala Phe Glu Asn Tyr Leu 1335
 ATT GGA CAT GTA GGC AAT TTG CTG GAC AGT GAC TCG GAA TTG CAT GCA ATT TAT 5121
 Ile Gly His Val Gly Asn Leu Leu Asp Ser Asp Ser Glu Leu His Ala Ile Tyr 1353
 AAT ATT ACT CCC CAA TCC ATT TCC ACA GAT ATT AAT ATT ATA ACG ACT CCA TTT 5175
 Asn Ile Thr Pro Gln Ser Ile Ser Thr Asp Ile Asn Ile Ile Thr Thr Pro Phe 1371

TAC	GAT	AAT	TCG	GGA	ACA	ATT	TAT	TCA	CCT	ACG	GTT	TTT	AAT	TTG	TTT	AAT	AAC	5229
Tyr	Asp	Asn	Ser	Gly	Thr	Ile	Tyr	Ser	Pro	Thr	Val	Phe	Asn	Leu	Phe	Asn	Asn	1389
AAT	TCC	CAT	GTC	GAT	GCA	ATG	AAT	TC										5255
Asn	Ser	His	Val	Asp	Ala	Met	Asn											1397

SEQ ID no. 1 B

Length of sequence:

1,188 pairs of bases

Type of molecule sequenced:

genomic DNA

Origin of the molecule:

Marek's disease virus, strain

RBI B

Experimental source:

pMDV 05 plasmid

Characteristics:

from 1 to 324 pairs of bases: non-coding region
from 325 to 1,135 pairs of bases: US1 gene: the gene is
transcribed from right to left; the
indicated sequence is complementary to
the sequence SEQ ID no. 1

Met Gly Val Ser
GAGATCAGACCGGCCGAGTTATTAAACAATAAAAAAGATTATTGGTGGAGTAGAG,ATG GGT GTG TCC 1189

Met Ile Thr Ile Val Thr Leu Leu Asp Gly Cys Asp Arg Leu Pro Gly Arg Ser
ATG ATA ACT ATA GTC ACA CTT CTA GAT GAA TGC GAT CGA TTG CCA GGA AGA TCT 1113

Arg Asp Ala Ala Ser Thr Leu Thr Trp Ile Phe Leu Ile Lys Gln Cys Met Glu Gln
AGA GAT GCT GCA TCT ACT TTA TGG ATA TTC CTT ATA AAG CAA TGT ATG GAA CAA 1069

Ile Gln Asp Asp Val Gly Val Pro Ile Ile Val Arg Ala Ala Asp Leu Phe Arg
ATA CAG GAT GAT GTG GGT GTG CCC ATA ATC GTC AGA GCT GCA GAC CTA TTC CGT 1015

Phe Ala Lys Pro Met Leu Ile Leu Pro Arg Gln His Arg Pro Ile Val Arg Thr
TTT GCC AAA CCC ATG TTA ATT CTT CCT CGG CAA CAT CGA CGS ATA GTA AGG ACA 961

Lys Pro Pro Asp Gly Thr Gly Val Arg Gly Thr Gly Leu Ala Gly Thr Arg Asp
AAG CCA CCA GAT GGA ACT GGA GTT CGT GGT ACC GGA TTG GCC GGA ACT AGS GAT 907

Ser Phe Ile Val Arg Leu Phe Gly Asp Val Ala Gly Cys Ser Thr Gly Trp Gln
TCG TTT ATA GTG CGG CTA TTT GAA GAT GTT SCA GGA TGT TCC ACA GAA TGG CAG 853

Asp Val Leu Ser Gly Tyr Leu Met Leu Gly Ser Gly Val Ser Gly Asn Ala Pro
GAT GTT CTA TCT GGA TAT TTG ATG TTG GAA TCT GAA GTT TCT GGT AAT GCT CCA 799

His Ser Leu Thr Ile Val Gly Ala Ala Asp Ile Cys Arg Ile Ala Leu Gly Cys
CAT AGC TTG TGG ATA GTT GGG GCG GCA GAT ATA TGT CGC ATT GCG CTC GAA TGT 745

Ile Pro Leu Pro Lys Arg Leu Leu Ala Ile Lys Val Ser Gly Thr Trp Ser Gly
ATT CCT TTG CCA AAA AGS TTA CTT GCA ATC AAA GTG TCT GGG ACC TGG TCC GGT 691

Met Pro Trp Ala Ile Pro Asp Asn Ile Gln Thr Leu Leu Thr Ser Thr Trp Gly
TGC CCG TGG GCC ATT CCC GAC AAT ATT CAA ACT CTC TTG ACA TCT ACA TGG GAA 637

Pro Lys Phe Asp Thr Pro Gly Asp Arg Ala His Phe Cys Asp Ser Asp Met Val
CCG AAG TTC GAC ACC CCA GAA GAT AGA GCG CAT TTT TGC GAC AGT GAT ATG GTA 583

Cys Val Tyr Lys Ile Leu Gly Ser Pro Pro Asn Pro Leu Lys Pro Pro Gly Ile
TGT GTA TAC AAA ATC CTC GGG TCC CCA CCC AAT CCT CTA AAA CCT CCG GAA ATC 529

Gly Pro Pro Gln Met Ser Ser Thr Pro Gly Arg Leu Phe Cys Cys Gly Lys Cys
GAA CCA CCT CAA ATG AGT AGT ACA CCC GGC AGA TTA TTC TGT TGT GGA AAA TGT 475

Cys Lys Lys Gly Asp Arg Asp Ala Ile Ala Ile Pro Val Arg Tyr Thr Ala Thr
TGC AAG AAA GAA GAT AGA GAT GCG ATT GCA ATT CCG GTT CTT TAC ACT GCG ACA 367

Gly Lys Ser Arg Ile Gln Lys Lys Cys Arg Ala Gly Ser His
GGA AAG TCA CGA ATA CAG AAA AAA TGT AGA GCC GGT AGT CAT TAGCTGTATTGCAC 310

AGACCTACTTCTACCAATTAGATATAATTAGATGATGGGGCGTATACACATTACGATTAGTGCATCGCTA 238

CAACCGTGGCTATAGTGTCACTGATATAATTTGTATATCTGCAATAACAAACCTTCTAGATCACTTATGTA 166

TCCAGGCTATCTTCATATACTTCTCAACATCAGGAGAGATTCAACAATCGAGCGCATTTGAAAGACAACGAT 94

GAGCAGAGTCAATGCTCAACATGTTGATGATATGATATACCAAGAGGACGATTTGGTAAAGCCAGCGAAA 22

GATTACTAATGTAAATTTTGG

SEQ ID no. 2

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' GGA CTC GAA CCA TGG AGT CTA TTA CC 3'
NCO 1

SEQ ID no. 3

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' GAC GGG TGT CGA CAT ATG AG 3'
SaiI

SEQ ID no. 4

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' CTG GAG CCC ATG GTG CAC CTT TG 3'
NCO1

SEQ ID no. 5

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' CAA ATT GCT ATT GTC GAC ACC TCC GCC TCT C 3'
SaiI

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE
PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Nucleotide sequence, and its variants, from the unique short domain (Us) of the virus of Marek's disease, characterized in that it corresponds to the US3 gene, is non-essential to replication, and is homologous with the kinase protein gene of the herpes simplex virus.
2. Nucleotide sequence US3 of the MDV virus, and his variants, according to claim 1 appearing on the sequence ID no. 1.
3. Nucleotide sequence ID no. 1 and genes and their variants including this sequence.
4. Recombinant virus selected from the herpes virus, comprising at least one heterologous gene inserted into the Us region of the genome of said virus corresponding to the gene of the kinase protein.
5. Recombinant virus according to claim 4, characterized in that the gene is a coding gene for a viral, bacterial or parasitic immunogene.
6. Recombinant virus of Marek's disease according to claim 5, comprising at least one heterologous gene, characterized in that this gene is inserted into the region of its genome

corresponding to the US3 gene, in such a way so as to be able to be expressed.

7. Recombinant virus according to claim 6, characterized in that the inserted heterologous gene codes for a pathogen selected from the group consisting of infectious avian bronchitis, Newcastle's disease, Gumboro's disease, fowl plague, chicken anaemia, egg-drop syndrome, fowl pox, infectious laryngo-tracheitis, avian coli bacillosis, pasteurelosis, coccidiosis, haemophilosis.

8. Recombinant virus according to one of claims 6 and 7, characterized in that this is the MDV virus.

9. Recombinant virus according to one of claims 6 and 7, characterized in that this is the HVT virus.

10. Recombinant virus according to claim 9, characterized in that the inserted gene codes for an immunogene of a Marek's disease virus of serotype 1 or 2.

11. Recombinant virus according to any one of claims 4 to 10, characterized in that the gene is inserted in order to be expressed under the control of the transcription regulating sequences of the gene of the kinase protein.

12. Recombinant virus according to one of claims 4 to 11, characterized in that the heterologous gene inserted is likely to be expressed under the control of promoting sequences of the virus in question, or other herpes viruses.

13. Recombinant virus according to any one of claims 4 to 12, characterized in that the start and stop codons of the gene inserted are substituted for those of the US3 gene.

14. Vaccine characterized in that it comprises a recombinant virus according to any one of claims 3 to 9.

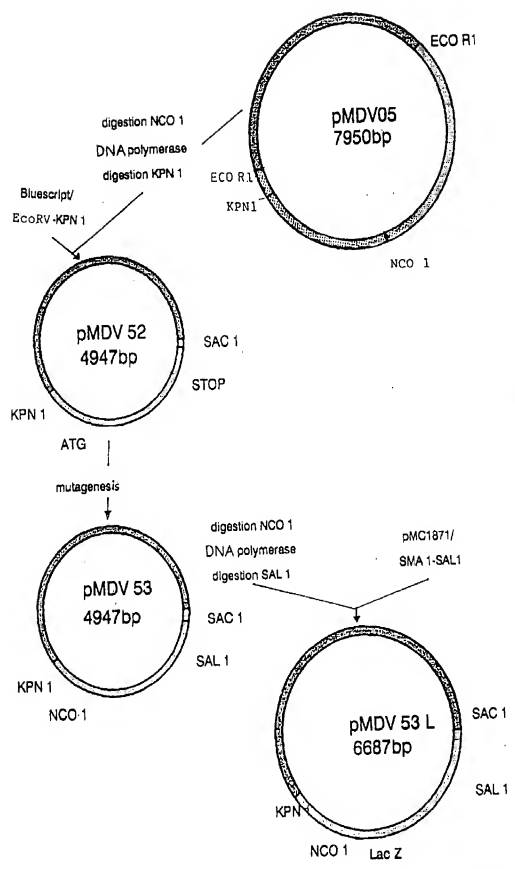
15. Process for preparation of a recombinant virus of Marek's disease, characterized in that at least one heterologous gene is inserted into the region of its genome corresponding to the US3 gene, in such a way so as to be able to be expressed.

FETHERSTONHAUGH & CO.
OTTAWA, CANADA

PATENT AGENTS

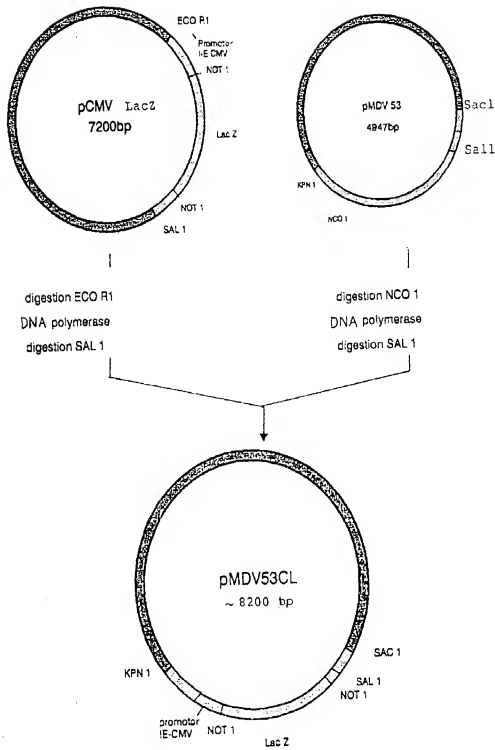
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Figure 1/3



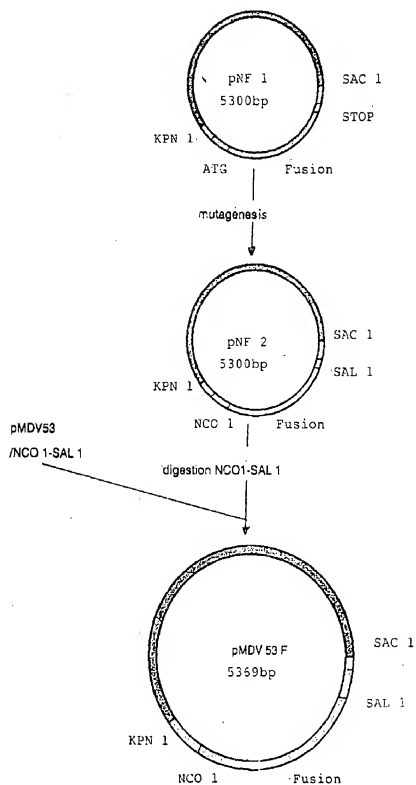
*Patent Application
Fellman, et al.*

Figure 2 / 3



Patent Pending
2000/01/01

Figure 3/3



Patent 10-10
27/10/2000